REMARKS/ARGUMENTS

By the present amendment, claims 8 and 12 have been amended and claims 20-47 have been deleted rendering claims 1, 4-10, 12-16, 18 and 19 pending in the application. The amendments to the clams have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicant reserves the right to pursue any of the deleted subject matter in a further continuation, continuation-in-part or divisional application. The amendment does not contain new matter and its entry is respectfully requested.

The Official Action dated September 17, 2003 and the Advisory Action have been carefully considered. It is believed that the amended claims submitted herewith and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Applicant thanks Examiner Steadman and Examiner Prouty for the interview held at the United States Patent Office on February 3, 2004.

Claim Objections

Claim 12 has been amended as requested by the Examiner.

35 USC §112, Second Paragraph

The Examiner has objected to claim 8 under 35 USC §112, second paragraph. In response, claim 8 has been amended in order to replace the article "A" with "The" as requested by the Examiner in the last office action.

In view of the foregoing, we respectfully request that the objection to claim 8 under 35 USC §112, second paragraph be withdrawn.

35 USC §102

The Examiner has rejected claims 20, 25, 26, 28-30, 41, 43 and 44 under 35 USC §102(b) as being anticipated by Hiramatsu et al. (*Appl Environ Microbiol* 56:2125-2132, 1990). By the present amendment, these claims have been deleted, without prejudice, which overcomes the objection.

In view of the foregoing, we respectfully request that the objection to the claims under 35 USC §102(b) be withdrawn.

35 USC §103

The Examiner has rejected claims 1, 4, 6-10, 13-16 and 19 under 35 USC §103(a) as being unpatentable over Hiramatsu et al. (*Appl Environ Microbiol* 56:2125-2132, 1990) in view of Hiramatsu et al. (*J Biol Chem* 264:16862-16866, 1989). We respectfully disagree with the Examiner for the reasons that follow.

The present invention relates to improved methods and compositions for preparing recombinant proteins in host cells. Prior to the present invention, it was determined that it was desirable to express recombinant proteins as a fusion protein in order to overcome a number of problems. One problem was that overproduced polypeptides can aggregate in the host cell in insoluble fractions known as inclusion bodies. The conversion of this insoluble material involves often slow and complex refolding methods, making protein purification difficult. Another problem was that proteins which are present in soluble form in the cytoplasm often are subject to degradation by host specific enzymes, thus reducing the amounts of active protein that can be recovered. Linking the polypeptide of interest to a fusion partner has been found to limit these problems. However, in order to recover the active polypeptide it is generally necessary to separate the fusion partner from the polypeptide of interest. Currently this is achieved through either an enzymatic means (e.g. by the addition of proteolytic enzymes) or chemical means (e.g. the addition of cyanogenbromide). Chemical cleavage often requires elevated temperature and toxic compounds which denature the recombinant protein and complicate purification. The prior art cleavage methods have been recognized to be either inefficient or lack cleavage specificity.

The present invention overcomes the problems of the prior art by providing compositions and methods for the **inexpensive, readily available** and **efficient** cleavage of recombinant fusion proteins. The method of the pending claims involves preparing the desired recombinant protein as a fusion protein with a chymosin pro-peptide sequence which is located immediately upstream of the recombinant protein without the mature form of the chymosin intervening. Upon the addition of a mature aspartic protease, the recombinant protein is efficiently cleaved from the pro-peptide. The novelty of the claimed invention is supported by the publication of the technology in a peer-reviewed journal, Protein Engineering, a copy of which we enclose. None of the prior art cited by the Examiner, either alone or in combination, would lead one of skill in the art to the present invention.

As the Examiner is aware, he has the burden of factually supporting any *prima facie* conclusion of obviousness. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, the prior art references when combined must teach or suggest all the claim limitations. Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Finally, there must be a reasonable expectation of success. We respectfully submit that the above criteria have not been met as explained below.

1) Prior art does not teach all claim limitations

Hiramatsu et al. (1990) and Hiramatsu et al. (1989) do not teach all of the claimed limitations.

First, the cited references do not teach a method for the preparation of a recombinant polypeptide wherein a mature form of an autocatalytically maturing aspartic protease is added to a fusion protein comprising a chymosin pro-peptide sequence linked to a heterologous protein. Second, the cited references do not teach that a chymosin pro-peptide can be efficiently cleaved from the fusion protein. Hiramatsu et al. (1990) is the

only reference that teaches a fusion protein comprising part of a pro-sequence linked to a heterologous protein. In particular, Hiramatsu et al. (1990) teaches only one vector JGH2 that contains part of a pro-sequence of Mucor Pusillus Rennin (MPR). The JGH2 vector comprises the full length pre-sequence and only five amino acids of the pro-sequence fused to human growth hormone via a 3 amino acid linker sequence. Hiramatsu et al. (1990) does not add a mature form of an aspartic protease to achieve cleavage. Importantly, Hiramatsu (1990) reports that they did not get efficient cleavage of the 5 amino acids of the pro-sequence from the hGH sequence with only part of the pro-sequence. In particular, they found that cleavage had occurred at the junction of the pre- and pro-sequences thus yielding an hGH sequence with 5 amino acids of the pro-sequence still N-terminally attached thereto. Therefore, Hiramatsu et al. (1990) did not achieve the present invention wherein the pro-peptide sequence is completely cleaved from the recombinant polypeptide. In this regard, we direct the Examiner to part (c) of claim 1 wherein it is stated that "the chymosin pro-peptide is cleaved from the fusion protein to release the recombinant polypeptide".

Hiramatsu et al. (1989) clearly does not teach the claimed limitations as it is not directed to the preparation of recombinant proteins and the use of a pro-chymosin fusion protein to achieve efficient cleavage of the recombinant protein.

2) No suggestion or motivation in the prior art

Hiramatsu et al. (1990) or Hiramatsu et al. (1989) provide no motivation to one of skill in the art to modify their teachings in order to achieve the present invention.

Hiramatsu et al. (1990) realized that it is undesirable to have part of the pro-peptide linked to the desired recombinant protein and expressly stated that:

> "Although **removal of the extra amino acids of the pro-peptide is required** to obtain hGH with the same NH_2-terminus as native hGH, it may be achieved by the **introduction of an artificial process site** ... just before the hGH sequence" (emphasis ours).

The Examiner states that the introduction of a cleavage site is only a suggestion by Hiramatsu et al. We strongly emphasize that it is the only suggestion offered by Hiramatsu and the only solution tried by Hiramatsu. In this regard, in their later publication, (Hiramatsu et al., *Applied and Environmental Microbiol*, Vol. 57, No. 7, p. 2052-2056, July 1991), Hiramatsu introduced an artificial linker that is recognized by the yeast KEX2 protease into their vectors in order to get efficient cleavage.

Therefore, while Hiramatsu et al. recognized the problem in the prior art, they provide no motivation to one of skill in the art to solve the problem by the method of the present invention. One of skill in the art having read Hiramatsu's work would in no way be lead to solve the problem by the method of the present invention. We respectfully submit that if the solution taught by the present invention was "obvious", then it at least would have been suggested in one of Hiramatsu's publications as they were clearly looking for a solution to the problem. It is important to note that Hiramatsu et al. does not add a mature form of an aspartic protease to effect cleavage of the pro-peptide as is specified in step (c) of pending claim 1.

The deficiencies in Hiramatsu et al. (1990) are clearly not remedied by Hiramatsu et al. (1989) as Hiramatsu et al. (1989) is merely describing the expression and cleavage of wild-type pre-pro chymosin. The fact that the pro-sequence is efficiently cleaved from the chymosin is not unexpected as that is how zymogens function. However, Hiramatsu et al. (1989) would provide no motivation or suggestion to one of skill in the art to use the pro-peptide sequence of chymosin for the efficient expression and recovery of a recombinant protein. This is again evidenced by the later Hiramatsu et al. publications (1990 and 1991) wherein they expressed a need for an artificial cleavage site and then go on to prepare such a construct without suggesting any alternate solutions.

3) No reasonable expectation of success

Neither of the Hiramatsu et al. references would provide one of ordinary skill in the art a reasonable expectation of success in achieving the present invention. Even if a person of ordinary skill in the art would be motivated to add the mature form of the

autocatalytically maturing aspartic protease (e.g. chymosin), there are several reasons as to why a person of ordinary skill in the art would not have a reasonable expectation of success.

First, if a person of ordinary skill in the art was compelled to add a mature aspartic protease (e.g. chymosin) to a fusion protein to facilitate cleavage, the cleavage site that such a person would likely expect to be cleaved is the protease's natural substrate. Chymosin is known to cleave bovine κ-casein at the Phe_{105}-Met_{106} bond in the coagulation of milk. The natural substrate for chymosin is κ-casein with a cleavage site consisting of the following amino acids – "RHPHPHLSF_{105}↓M_{106}AIPPKKG" (the "κ-casein recognition site"). The fusion polypeptide provided in accordance with the present invention does not include a κ-casein recognition site. In order to further support this position Applicant herewith encloses, Walsh and Swaisgood (1996) J. Biotech 45: 235-241. The authors of this paper, likely not individuals of *ordinary* skill but rather advanced skill in the art, were faced with essentially the same problem as the present inventors prior to the conception of the present invention: i.e. how does one effectively cleave a fusion protein. The authors of this paper then conceived of the use of chymosin as a means to achieve such cleavage, however they designed their fusion polypeptide to include a κ-casein recognition site Thus a person of ordinary skill would not expect success if they would add the mature chymosin to the chymosin pro-sequence as the pro-sequence if structurally different from the κ-casein recognition site.

A second reason why a person of ordinary skill in the art would not have a reasonable expectation of success is that proteases frequently are not specific enough and would cleave in multiple locations in the fusion protein of interest (i.e. trypsin cleaves after all lysine residues) resulting in a plurality of peptide fragments rather than a separation into the desired product and the fusion partner. Thirdly, cleavage may result in several foreign amino acid residues attached to the protein of interest. This is illustrated with the use of enzymes like thrombin which cleave the sequence Leu-Val-Pro-Arg↓Gly-Ser and would result in either Gly-Ser residues being attached to the N-terminus of the

protein of interest or Leu-Val-Pro-Arg residues being attached to the C-terminus of the protein of interest. Other examples are found in Table 1 below. The addition of foreign amino acids to the peptide of interest would alter the properties of the protein.

Table 1: Exemplary proteases and their respective excision sites.

Enzyme	Excision site
Factor Xa	Ile-Glu/Asp-Gly-Arg↓
Thrombin	Leu-Val-Pro-Arg↓Gly-Ser
Enterokinase	Asp-Asp-Asp-Asp-Lys↓
Renin*	Pro-Phe-His-Leu↓Leu-Val-Tyr (Haffery (1987))
Collagenase	Gly↓Ile/Leu

** Renin is an endopeptidase from the submazillary glands of mice. This is different from the milk clotting enzyme chymosin or rennin.*

LaVallie et al. (1994) "Enzymatic and chemical cleavage of fusion proteins" *In* Current Protocols in Molecular Biology pp 16.4.5-16.4.17, John Wiley and Sons, Inc, New York NY (copy enclosed). provides guidance for the cleavage of fusion proteins to remove the carrier. As above, LaVallie et al. has identified that multiple proteases leave extra amino acids on the protein of interest and has suggested using Factor Xa and enterokinase because these enzymes cleave on the carboxy-terminal side of their respective recognition sequences, allowing the release of fusion partners containing their authentic N-termini. It should be noted however success is not guaranteed because there are limitations as to which amino acids can be C-terminal to the cleavage site for Factor Xa or Enterokinase. For example, Factor Xa is inhibited by adjacent hydrophobic sequences of the C-terminal side of the potential cleavage site (see He et al., J Protein Chem 1993 12(1):1-5, copy enclosed). Finally, success is also not guaranteed when using a protease to cleave off the protein of interest as proteases are often inefficient so that yields of the cleaved protein are significantly decreased. As identified by Johnston and Dougherty (US patent 5,532,142, Column 1, lines 48-54,

copy enclosed)) the inefficiency is often due to impurities that may either partially inactivate the protease or the protease itself may have non-selective proteolytic activity.

In summary, we respectfully submit that the Examiner has not met the burden of establishing obviousness in the present case as none of the above criteria have been satisfied.

We remind the Examiner that he is to consider secondary considerations when assessing obviousness. Secondary considerations relevant to the present invention include long-felt need in the art. The expression of valuable recombinant proteins (such as therapeutic proteins) is highly desirable. However, as stated previously, there are difficulties recognized the art with respect to the efficient production and recovery of recombinant proteins. The present invention solves the difficulties of the prior art by providing an efficient method of cleaving recombinant proteins. Further, the present invention permits the use of fusion proteins for larger scale manufacturing, both by reducing the cost of the cleavage agent and by addressing safety issues arising from the use of certain cleavage agents. Therefore, there is a clear long felt need in the art for the present invention which must be given due weight when considering inventive step.

The Examiner has rejected claim 5 under 35 USC §103(a) as being unpatentable over Hiramatsu et al. (1990) in view of Hiramatsu et al. (1989) as applied to claims 1, 4, 6-10, 13-16 and 19 above and further in view of Fine et al. (*Gen Comp Endocrinol* 89:51-61) and the rejection of claim 24 as being unpatentable over Hiramatsu et al. (1990) in view of Fine et al. We respectfully disagree with the Examiner for the reasons that follow.

Claims 5 and 24 relate to specific embodiments of the invention wherein the heterologous protein is carp growth hormone. As a result, these claims carry with them all of the novel and inventive features of claims 1 and 20 from which these claims depend. Our comments on the Hiramatsu et al. references appear above and equally apply to these claims. The deficiencies in the Hiramatsu et al. references are in no way

remedied by Fine which is a reference that describes expression of Carp Growth Hormone (cGH) in *E.coli*, purification of cGH from *E.coli*, *in vitro* (using lymphoma and preadipocyte cells) and *in vivo* (evaluating growth rate in fish injected with the purified protein) characterization of cGH. Fine in no way teaches or suggests an improved method to prepare cGH by linking the cGH to a pro-peptide from chymosin.

In view of the foregoing, we respectfully request that all of the objections under 35 USC §103 be withdrawn.

The Commissioner is hereby authorized to charge any deficiency in fees (including any claim fees) or credit any overpayment to our Deposit Account No. 02-2095.

In view of the foregoing, we submit that the application is in order for allowance and an early indication to that effect would be greatly appreciated. Should the Examiner like to discuss the matter, he is kindly requested to contact Micheline Gravelle at 416-957-1682 at his convenience.

Respectfully submitted,

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Attachments